III. *On Rubian and its Products of Decomposition.* *By* EDWARD SCHUNCK, *F.R.S.*

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PART II.

Action of Alkalies and Alkaline Earths on Rubian.

IN the first part of this paper I have described, in general terms, the effect produced on rubian by alkalies. It is only the fixed alkalies that are capable of effecting the decomposition of rubian. Ammonia produces no perceptible change in a watery solution of this substance, except that of altering its colour from yellow to blood-red. This blood-red colour remains unchanged even after long-continued boiling, and the solution still contains rubian, for on supersaturating the ammonia with acid, the solution again becomes yellow, and not the slightest precipitate is produced. The fixed alkalies, on the other hand, act very differently. On adding caustic soda to a solution of rubian, the colour of the solution first changes to blood-red. On boiling the liquid, however, its colour soon changes again from blood-red to purple. This alteration in colour indicates the formation of alizarine. If the boiling be continued, there is deposited, as the liquid becomes more concentrated, a dark purple powder, which consists mainly of a compound of alizarine and soda, and separates in consequence of its insolubility in caustic lye. After the liquid has been boiled for some time, then, provided the quantity of soda employed be sufficiently large, the rubian is entirely decomposed. On now adding sulphuric acid in excess, a quantity of orange-coloured flocks, exactly like those produced by the action of acids on rubian, is precipitated, while the liquid becomes almost colourless. These flocks are separated by filtration and washed with cold water, until the sulphate of soda and sulphuric acid are entirely removed. They now consist mainly of four substances, viz. 1st, *Alizarine*; 2ndly, *Rubiretine*; 3rdly, *Verantine*; and 4thly, a substance which has not hitherto been observed, and to which I shall give the name of *Rubiadine*.

In order to separate these substances from one another, I adopt almost the same method as that employed for the separation of the products of decomposition with acids. The mixture is first treated with boiling alcohol, which dissolves the greatest part, leaving undissolved however a considerable quantity of a dark brown flocculent substance. This substance invariably accompanies the other products of the action. It is, however, as its properties and composition show, a secondary product of decomposition, the formation of which I shall explain presently. To the dark yellow alcoholic solution, after filtration from this flocculent substance, there is added acetate of alumina, which produces a dark red precipitate. This precipitate,

which is a compound of alizarine and verantine with alumina, is decomposed with muriatic acid, and the alizarine and verantine are separated from one another by means of acetate of copper, and purified in the manner which I have before described, when treating of the products of decomposition with acids. The liquid filtered from this alumina compound is still yellow. On adding to it sulphuric acid and a large quantity of water, the substances dissolved in it are precipitated in the shape of yellow flocks, which after filtration and edulcoration are redissolved in boiling alcohol. On adding acetate of lead to this solution, there is produced a dark purplish-brown precipitate, which consists of rubiretine and verantine in combination with oxide of lead. The liquid still remains yellow, and is filtered from the precipitate. The latter, on being decomposed with boiling muriatic acid, gives a brown powder, which, after filtration and edulcoration, is treated with cold alcohol. This dissolves the rubiretine, leaving behind the greatest part of the verantine. The alcoholic liquid, after filtration and evaporation, leaves a residue of rubiretine with its usual appearance and properties. Should it not melt easily when thrown into boiling water, it must again be treated with a small quantity of cold alcohol, when an additional portion of verantine remains undissolved, and the alcohol on evaporation usually gives pure rubiretine. Should it, however, still contain verantine, the process of treating with cold alcohol must be repeated, until it is free from that substance. The alcoholic liquid filtered from the lead precipitate contains the substance which I call rubiadine. In order to obtain it in a state of purity, water is added to the solution. This produces a yellow precipitate, which after filtration is treated with just sufficient boiling alcohol to dissolve it. To the solution while boiling, there is added hydrated oxide of lead, which takes up the impurity, consisting chiefly of rubiretine, and renders the solution of a lighter yellow. The liquid, after being filtered boiling hot, deposits the rubiadine on cooling in small yellow needles. An additional quantity may be procured by evaporating the mother-liquor, but this portion is not sufficiently pure to assume a crystalline form, and has the appearance of an amorphous yellow powder. By exposing it to heat, however, a crystalline sublimate of rubiadine may be obtained.

The acid liquid filtered from the mixture of the four preceding substances still contains in solution another product of decomposition. After neutralizing the sulphuric acid with carbonate of lead, and evaporating the filtered liquid to dryness, a residue is left, which is treated with alcohol. This leaves undissolved the sulphate of soda, and after filtration and evaporation gives a brown glutinous mass, which has all the properties of the sugar produced by the action of acids on rubian. It is this sugar, which, by the continued action on it of the caustic alkali, gives rise to the formation of the dark brown flocculent substance, insoluble in alcohol, which is found mixed with the four preceding products of decomposition. This fact is sufficiently proved by the composition of the latter substance, which is identical with that of the brown substance produced by the action of strong acids on ordinary sugar.

The products formed by the action of caustic baryta on rubian do not differ from those resulting from the action of caustic soda. On adding caustic baryta to a solution of rubian and boiling for some time, the rubian is entirely decomposed; the decomposition, as might be supposed, requiring a rather longer time for its completion than in the case of caustic soda. After the process is finished, the products of decomposition are found for the most part in combination with baryta in the shape of a purple powder, while the liquor still retains a red colour. The purple powder is placed on a filter and slightly washed with cold water. On passing carbonic acid through the filtered liquid, the latter loses its red colour, and a yellow flocculent substance is precipitated together with carbonate of baryta. The liquid, after being boiled in order to decompose the bicarbonate of baryta, and then filtered, is evaporated over sulphuric acid at the ordinary temperature, when it leaves a substance of a light brown colour, which resembles the sugar produced by the action of acids on rubian, but is not quite so deliquescent. This substance, as I shall presently show, is in fact a baryta compound of sugar. The mixture of yellow flocks and carbonate of baryta is now added to the purple powder before mentioned, and the whole is treated with muriatic acid in order to extract the baryta. The acid leaves undissolved a quantity of orange-coloured flocks, which consist, as before, of alizarine, rubiretine, verantine, and rubiadine, as well as of some of the dark brown flocculent substance, insoluble in boiling alcohol, and are separated from one another in the manner just described. If sulphuric acid be employed instead of carbonic acid for separating the baryta from the red liquid, and the excess of acid be neutralized with carbonate of baryta, the liquid on evaporation gives a substance exactly similar to that obtained in the other case, being also a compound of sugar and baryta, but differing from the latter in containing only half the quantity of baryta. The identity of the alizarine and rubiretine obtained by the action of alkalies, with those produced by the action of acids on rubian, is proved by the following analyses.

0·2255 grm. crystallized alizarine obtained by means of caustic soda, lost on being heated in the water-bath, 0·0405 water =17·96 per cent.

0·1820 grm. of the dry substance, burnt with chromate of lead*, gave 0·4610 carbonic acid and 0·0770 water.

These numbers correspond in 100 parts to—

Carbon	69·07
Hydrogen	4·70
Oxygen	26·23

I. 0·2610 grm. rubiretine, prepared by means of caustic soda, and dried at 100° C., gave 0·6565 carbonic acid and 0·1250 water.

* In order to avoid repetition, I may state that all the organic analyses given in this paper, in which the material used for combustion is not especially mentioned, were performed with chromate of lead.

II. 0·3630 grm. rubiretine, prepared by means of caustic baryta, gave 0·9130 carbonic acid and 0·1715 water.

These numbers correspond in 100 parts to—

	I.	II.
Carbon	68·60	68·59
Hydrogen	5·32	5·24
Oxygen	26·08	26·17

I did not obtain a sufficient quantity of verantine in a pure state for the purpose of analysis, but the properties of the substance formed by the action of alkalies on rubian corresponded so exactly with those of the verantine produced by the action of acids, as to leave no doubt of their identity.

The compound of sugar and baryta, obtained by the action of baryta on rubian, is similar in appearance to the sugar itself. Its colour is light brown, and when dry it is brittle and transparent like dried gum or varnish. It exhibits no trace of crystalline form. It is more easily reduced to a state of dryness than the sugar itself, and deliquesces much less when exposed to a moist atmosphere than the latter. When its solution is mixed with acetate of lead, the whole of the sugar seems to be precipitated in combination with oxide of lead. On analysing a specimen of this substance prepared by precipitating the excess of baryta with carbonic acid, I obtained the following results:—

I. 0·6375 grm. heated for several hours in the water-bath, gave 0·5245 carbonic acid and 0·1945 water.

II. 0·7575 grm. gave 0·6320 carbonic acid and 0·2235 water.

0·4860 grm. gave 0·3480 sulphate of baryta, equivalent to 0·22838 baryta.

From these numbers may be deduced the following composition:—

	Eqs.		Calculated.	I.	II.
Carbon	12	72	22·20	22·43	22·69
Hydrogen . . .	11	11	3·39	3·39	3·26
Oxygen	11	88	27·14		27·06
Baryta	2	153·2	47·27		46·99
		324·2	100·00		100·00

If the baryta contained in the solution of sugar be precipitated with sulphuric acid, and the latter be again neutralized with carbonate of baryta, there is obtained, as I mentioned above, a compound of sugar and baryta containing less baryta than the preceding. Its analysis yielded the following results:—

I. 0·4330 grm. gave 0·4670 carbonic acid and 0·1825 water.

II. 0·3770 grm. heated for several hours longer at 100° C., gave 0·4150 carbonic acid and 0·1570 water.

0·3890 grm. gave 0·1890 sulphate of baryta, equivalent to 0·1240 baryta.

Hence may be deduced the following composition:—

	Eqs.		Calculated.	I.	II.
Carbon	12	72	30·17	29·41	30·01
Hydrogen . . .	10	10	4·19	4·68	4·62
Oxygen	10	80	33·54		33·49
Baryta	1	76·6	32·10		31·88
		238·6	100·00		100·00

This compound, it will be seen, is identical in composition with the baryta compound of cane sugar. If the formulæ of the two compounds be compared together, it is evident that if both be correct, the one containing most baryta must be written thus,—$C_{12} H_{10} O_{10} + BaO + BaO\ HO$. In this case, however, it would be difficult to conceive how it is possible for the second atom of baryta to escape the action of the carbonic acid used in its preparation. It is therefore more probable that the true formula is $C_{12} H_{10} O_{10} + 2BaO$, which requires in 100 parts—

Carbon	22·84
Hydrogen	3·17
Oxygen	25·39
Baryta	48·60

In fact the amount of carbon and hydrogen found by experiment agree better with these numbers than with those of the other formula.

These analyses lead to the conclusion, that the sugar formed by the decomposition of rubian contains 12, not 14 atoms of carbon. Nevertheless it is doubtful whether in these baryta compounds the sugar exists in the same state as it does when formed by the action of acids on rubian. In the latter state it seems incapable of entering into combination with bases such as baryta or oxide of lead. It is therefore probable that by the continued action on it of alkalies or alkaline earths it undergoes some change, possibly in consequence of the loss of the elements of water.

Among the products of the action of alkalies on rubian, I have mentioned a dark brown substance insoluble in boiling alcohol. This substance is soluble in alkalies with a brown colour, and is reprecipitated by acids. It resembles in all respects the black substances bearing the general name of humus, which are formed by the action of strong acids and alkalies on ordinary sugar. It was analysed with the following result:—

0·3215 grm. gave 0·8040 carbonic acid and 0·1260 water.

In 100 parts therefore it contains—

Carbon	68·20
Hydrogen	4·35
Oxygen	27·45

The ulmic acid of Mulder, obtained by the action of muriatic acid on cane-sugar, contains, according to that chemist (C=6·11),—

Carbon	68·95
Hydrogen	4·23
Oxygen	26·82

The formula given by Mulder for ulmic acid, viz. $C_{40}\,H_{14}\,O_{12}$, requires in 100 parts—

Carbon	68·57
Hydrogen	4·00
Oxygen	27·43

The identity in composition and properties of this acid, and the substance formed by the action of alkalies on rubian, leave no doubt of their being the same.

Rubiadine.—This substance, which has not hitherto been observed among the products derived from madder, bears a close resemblance in its appearance and many of its properties to rubianine, the place of which it in fact occupies in the series of substances produced by the action of alkalies on rubian. Besides its composition, however, there are several properties belonging to it so characteristic, that it cannot be confounded with rubianine or any of the substances previously described. When crystallized from alcohol, it is obtained in the shape of small yellow or orange-coloured needles. A very minute degree of impurity, however, seems to prevent its assuming a crystalline form, in which case it is obtained in small granular masses, or as a yellow amorphous powder. It may be purified by dissolving it in a small quantity of boiling alcohol, and adding to the boiling solution either hydrated oxide of lead, or protoxide of tin. On filtering boiling hot and allowing to cool, it crystallizes out. When heated on platinum foil, it melts and burns with flame. When cautiously heated between two watch-glasses, it may be almost entirely volatilized. On the lower glass a very slight carbonaceous residue is left, while the upper glass is covered with a quantity of partly yellow, partly orange-coloured micaceous scales, endowed with considerable lustre. These scales possess all the properties of rubiadine itself. Rubiadine is insoluble in water. It communicates hardly any colour to boiling water, and the filtered liquid deposits nothing on cooling. It is more soluble in alcohol than rubianine. It dissolves in concentrated sulphuric acid with a dark yellow colour, and is reprecipitated by water in yellow flocks. If the solution in sulphuric acid be boiled, the colour changes to a dark yellowish-brown, a little sulphurous acid is disengaged, and the addition of water now causes a yellowish-brown precipitate. On treating rubiadine with boiling nitric acid, it dissolves, nitrous acid is disengaged, and the liquid on cooling deposits nothing, so that the substance seems to be decomposed by the acid. Towards alkalies rubiadine behaves in a similar manner to rubianine. Ammonia and carbonate of soda change its colour very little in the cold. It is only on boiling the alkaline liquids that it dissolves with a blood-

red colour. It is precipitated from its alkaline solutions by acids in thick yellow flocks. The ammoniacal solution on exposure to the air loses its ammonia and deposits the substance in the shape of a yellow uncrystalline pellicle. On adding chloride of barium to the ammoniacal solution, no effect is produced at first, but after some time a slight dark red precipitate falls. Chloride of calcium, added to the ammoniacal solution, produces almost immediately a copious light red precipitate. Acetate of lead gives no precipitate in an alcoholic solution of rubiadine, and water throws down rubiadine in an uncombined state. On adding acetate of copper to the alcoholic solution, no effect ensues at first beyond a darkening of the solution, but after a few moments a dark brownish-red precipitate falls; the supernatant liquid remains yellow, but contains very little rubiadine in solution. When treated with a boiling solution of perchloride of iron, rubiadine does not dissolve. The liquid changes very slightly in colour, and gives after filtration only a very slight precipitate on the addition of muriatic acid. The residue left undissolved by the perchloride of iron is unchanged rubiadine. By its volatility, when exposed to heat, and by its insolubility in boiling water, rubiadine may be easily distinguished from rubianine, which cannot be strongly heated without decomposition, and is soluble in boiling water.

I only obtained sufficient rubiadine in a state of purity for one analysis, which gave the following results:—

0·2575 grm. gave 0·6725 carbonic acid and 0·1120 water.

These numbers correspond in 100 parts to—

Carbon	71·22
Hydrogen	4·83
Oxygen	23·95

There are several formulæ with which this composition agrees, but only two which at the same time satisfactorily explain the formation of this substance, viz. $C_{32}H_{12}O_8$ and $C_{44}H_{15}O_{11}$. The great similarity in properties between rubiadine and rubianine makes it probable that both have a similar composition, and as it is almost certain from the experiments above detailed that the formula of the sugar resulting from the decomposition of rubian is $C_{12}H_{12}O_{12}$, it follows that the true formula of rubiadine must be one of the two just given.

These formulæ require respectively in 100 parts—

	$C_{32}H_{12}O_8$.	$C_{44}H_{15}O_{11}$.
Carbon	71·64	71·93
Hydrogen	4·47	4·08
Oxygen	23·89	23·99

If the first formula be the correct one, then the formation of rubiadine is due to the splitting up of 1 equiv. of rubian, after the assimilation of 2 equivs. of water, into 1 equiv. of rubiadine and 2 equivs. of sugar, as will be seen by the following equation:—

$$\left.\begin{array}{ll}\text{2 equivs. of Sugar} & =C_{24}H_{24}O_{24}\\ \text{1 equiv. of Rubiadine} & =C_{32}H_{12}O_{8}\end{array}\right\}=\left\{\begin{array}{l}C_{56}H_{34}O_{30}=\text{1 equiv. of Rubian.}\\ H_2O_2=\text{2 equivs. of Water.}\end{array}\right.$$

$$\overline{C_{56}H_{36}O_{32}} \qquad \overline{C_{56}H_{36}O_{32}}$$

If the formula of rubianine be $C_{32} H_{19} O_{15}$, rubiadine will differ from it by containing the elements of 7 equivs. of water less. If the formula of rubiadine be $C_{44} H_{15} O_{11}$, then its formation would presuppose the splitting up of 1 equiv. of rubian into 1 equiv. of rubiadine, 1 equiv. of sugar and 7 equivs. of water, for

$$C_{44} H_{15} O_{11} + C_{12} H_{12} O_{12} + 7HO = C_{56} H_{34} O_{30}.$$

It appears therefore that the action of alkalies on rubian differs in its results from the action of acids only in one respect, that alkalies as well as acids give rise to the formation of five distinct products of decomposition, that four of these are in both cases perfectly identical, and that the fifth substance produced by the action of acids is replaced in the case of alkalies by another, which, though perfectly distinct, is so similar both in properties and composition, that it may be considered as in every respect its equivalent.

Action of Ferments on Rubian.—It has long been suspected by chemists that the colouring matter of madder owes its formation to some process of fermentation, but the exact nature of the process has hitherto remained unknown. Mr. HIGGIN first pointed out the fact, that a formation of colouring matter takes place even during the short period occupied in the process of dyeing, and he attributes it to the action of some albuminous substance contained in madder on xanthine. That some process of decomposition takes place on extracting madder with cold or tepid water and exposing the extract to a moderate temperature, is proved by the fact that the extract, if concentrated, becomes after some time thick and gelatinous; and that the process of decomposition takes effect chiefly on the rubian is apparent, since the extract, after it has become gelatinous, is found to have lost its bitter taste and the greater part of its yellow colour.

In order to prepare the peculiar substance contained in madder which has the property of effecting the decomposition of rubian, I proceed in the following manner. A quantity of madder having been placed on a piece of calico or fine canvas, there is poured on it for every pound of madder taken about four quarts of distilled water, which may be either cold or of a temperature of about 38° C. The latter temperature is preferable, as the water then percolates more rapidly. To the extract there is added, without any delay, about an equal volume of alcohol, which causes the separation of a quantity of dark reddish-brown flocks. These flocks are allowed to settle, and the supernatant yellowish-brown liquid is decanted. Fresh alcohol is added to the flocks, which are then placed on a paper filter, and washed on the filter with additional quantities of alcohol, until the percolating liquid, which is at first strongly coloured, becomes almost colourless. This process of washing, which often lasts a long time, may be assisted by removing the mass from the filter and shaking it up with moderately warm alcohol. The substance on the filter has now the appearance of a dark reddish-brown mass, which when rubbed between the fingers produces the same granular feeling as coagulated caseine, the sliminess which characterized it on its first precipitation with alcohol having disappeared. Now this substance possesses

in an eminent degree the power of effecting the decomposition of rubian. If a quantity of it be added to a solution of rubian, and the mixture be left to stand at the ordinary temperature, a complete change is found to have taken place in the course of a few hours. The liquid is converted into a trembling jelly of a light brown colour, which is perfectly tasteless, insoluble in cold water, and so consistent, that if the solution of rubian was tolerably concentrated, the vessel may be reversed without its falling out. It is manifest from this experiment that it is this substance which constitutes the active fermenting principle of madder, and that for the purpose of understanding the nature of the process of fermentation peculiar to madder, it is necessary to examine the action of this substance on rubian. I shall therefore in the first place describe the products resulting from this action, after which I shall return to the consideration of its other properties.

On adding to a watery solution of rubian a quantity of the ferment, prepared in the manner just described, the latter loses its granular nature and diffuses itself in the solution without actually dissolving, forming a muddy, mucilaginous liquid. On allowing this mixture to stand in a moderately warm place, the rubian is gradually decomposed and converted into a brown jelly, similar in appearance and consistency to coagulated blood. Here and there interspersed in the mass yellow streaks and spots, consisting of long hair-like crystals, are sometimes discernible. If, after twenty-four hours, the jelly on being mixed with cold water still yields to the latter any yellow colour, it is a sign that the rubian is not entirely decomposed, and more ferment must be added, the mixture being again allowed to stand until the liquid has become tasteless and colourless. During this process none of the usual signs of fermentation are manifested. The liquid remains perfectly neutral, and no gas of any kind is disengaged. In order however to be perfectly sure of the latter point, I took a solution of rubian in water, mixed it with ferment, poured the mixture into a graduated glass tube, and inverted the latter over mercury. After standing in this way for several days, not a particle of gas had collected at the top of the tube, notwithstanding which the rubian was entirely decomposed, and on examination the products of decomposition were found to be precisely the same as those formed in the usual manner. This experiment proves not only that no gas is evolved during the fermentation of rubian, but also that, as HIGGIN has observed, the access of atmospheric air is not necessary during the process.

The decomposition of the rubian being completed, a little water is added to the mass, and the gelatinous substance left undissolved by the water is placed on a filter and slightly washed with cold water. The percolating liquid is almost colourless. The gelatinous mass on the filter now consists partly of the ferment employed, and partly of substances formed by the decomposition of the rubian. These substances are six in number, of which three are bodies previously described, and three are new. They are,—1st, *Alizarine*; 2ndly, *Verantine*; 3rdly, *Rubiretine*; 4thly, a substance closely resembling rubiacine, which I shall call *Rubiafine*; 5thly, a substance very

similar to rubianine and rubiadine, to which I shall give the name of *Rubiagine*; and, 6thly, a peculiar fatty substance which I shall denominate *Rubiadipine*. These substances are separated in the following manner. The mixture is treated with boiling alcohol. The alcohol, which assumes a dark reddish-yellow colour, is filtered, and the substance on the filter is treated again with boiling alcohol, until the latter acquires thereby only a light yellow colour. The substance left on the filter consists of the ferment, which has undergone no apparent change either in quantity or properties. To the alcoholic liquid there is now added acetate of alumina, which produces a dull yellowish-red precipitate. This precipitate, which consists of alizarine, verantine and rubiafine in combination with alumina, is separated by filtration and decomposed with boiling muriatic acid, which leaves undissolved a quantity of orange-coloured flocks. The liquid filtered from the alumina precipitate has a dark brownish-red colour. By adding to it sulphuric acid and a large quantity of water, the substances contained in it are precipitated in the shape of a yellow powder. This powder yields nothing to boiling water, which proves the absence of rubianine among the products of decomposition. After being separated by filtration, and washed with water to remove the acid and aluminous salts, it is again dissolved in boiling alcohol, and to the boiling solution there is added an excess of sugar of lead, which causes a dark purple precipitate, the liquid becoming dark yellow. The latter is filtered boiling hot and then mixed with a large quantity of water, which produces a dull orange-coloured precipitate, consisting of rubiagine and rubiadipine in combination with oxide of lead. This precipitate is boiled with sulphuric acid, which turns it yellow; and after the excess of acid has been removed with water, it is treated with boiling alcohol, which leaves undissolved a quantity of sulphate of lead, and acquires a yellow colour. The alcohol on evaporation leaves a residue consisting of rubiagine and rubiadipine. This residue is treated with cold alcohol, in which the rubiadipine dissolves easily, and is obtained on again evaporating as a dark brown, soft, fatty mass. The rubiagine left undissolved is treated with a small quantity of warm alcohol, which removes an additional quantity of rubiadipine, together with a portion of the substance itself, and on redissolving it in boiling alcohol and evaporating spontaneously, it appears in the form of a lemon-yellow mass consisting of small crystalline grains. The dark purple precipitate produced by sugar of lead consists of oxide of lead in combination with rubiretine, alizarine, verantine and rubiafine. It is treated with boiling muriatic acid, which dissolves the oxide of lead. The yellow flocks left by the muriatic acid are, after washing with water, treated with cold alcohol which leaves a part undissolved, and on evaporation gives a residue consisting for the most part of rubiretine. By treating this residue again with cold alcohol, an additional quantity of substance is left undissolved, and the rubiretine is now obtained in a state of greater purity. Its appearance and properties are the same as when obtained by the action of acids or alkalies on rubian. The substance left undissolved by the cold alcohol is added to the orange-coloured flocks proceeding from the

decomposition of the alumina precipitate, and the mixture is dissolved in boiling alcohol. To the alcoholic solution is added acetate of copper, which gives a dirty purple precipitate, consisting of verantine, rubiafine and a portion of the alizarine in combination with oxide of copper, while the liquid, which has a fine purple colour, contains the greatest part of the alizarine. The latter is obtained from this liquid by precipitating with muriatic acid and water, and purified by crystallization. The precipitate produced by acetate of copper is decomposed with muriatic acid, which leaves undissolved a quantity of red flocks. These, after filtration and washing with water, are dissolved in boiling alcohol. Into the boiling solution is introduced a quantity of hydrated protoxide of tin, which thereby acquires a light brown colour, while the liquid becomes light yellow, and on being filtered boiling hot and allowed to cool, deposits the rubiafine in yellow shining plates and needles, which are purified by recrystallization from alcohol. The oxide of tin-lake left on the filter is treated with cold muriatic acid, which dissolves the greatest part of the oxide of tin, and leaves undissolved a dark reddish-brown powder. This is placed on a filter, washed first with muriatic acid, then with water, and then treated with boiling alcohol. The alcohol leaves a great part of it undissolved, and on being filtered boiling hot deposits on cooling a brown powder consisting of verantine. The liquid on evaporation gives a quantity of alizarine, mixed with verantine. The substance left undissolved by the boiling alcohol is dark brown. It is a compound of verantine and oxide of tin, from which I have not been able to extract the oxide of tin by acids or any other means. It is soluble in ammonia and carbonate of soda, and is precipitated unchanged by acids.

The same products as those just mentioned may be obtained from madder by extracting it with cold or tepid water, allowing the extract to stand until a coagulum has been formed in it, placing the coagulum on a calico strainer and then treating it with boiling alcohol. The alcohol leaves undissolved a quantity of ferment, and is found to contain the same substances as those originating from the direct action of the ferment on rubian.

The liquid filtered from the gelatinous mixture of substances insoluble in water, formed by the action of the ferment, still contains in solution a considerable quantity of sugar. On adding to it a small quantity of caustic baryta, a pinkish-white precipitate falls, consisting probably of pectic acid in combination with baryta. The liquid being filtered, the baryta is precipitated with sulphuric acid, the excess of the latter is removed with carbonate of lead, and a small quantity of lead which dissolves is thrown down with sulphuretted hydrogen. The liquid now leaves, on evaporation at the ordinary temperature over sulphuric acid, a brownish-yellow syrup, having the same appearance and properties as the sugar produced by the action of acids on rubian.

The following analyses prove the identity of the alizarine formed by the fermentation of rubian with that derived from other sources:—

I. 0·5210 grm. alizarine, produced by the action of the ferment on rubian, lost, on being heated in the water-bath, 0·0920 water = 17·65 per cent.

0·2500 grm. of the dry substance gave 0·6380 carbonic acid and 0·0960 water.

II. 0·4220 grm., obtained by allowing an extract of madder with tepid water to ferment, lost, on being heated, 0·0750 water = 17·77 per cent.

0·3225 grm. of the dry substance gave 0·8230 carbonic acid and 0·1170 water.

These numbers correspond in 100 parts to—

	I.	II.
Carbon	69·59	69·59
Hydrogen	4·26	4·03
Oxygen	26·15	26·38

The quantity of rubiretine and verantine formed by the fermentation of rubian does not seem to be so large as it is, when acids or alkalies are employed for its decomposition, and as the process of separating and purifying the products of fermentation is, on account of the number of products formed, rather more complicated, the quantity of each substance obtained in a pure state is but small. The following analyses of these two substances were made with specimens obtained by the fermentation of an extract of madder; and even by preparing them in this manner I had some difficulty in procuring a sufficient quantity for examination, because the rubiretine formed by fermentation is always found mixed with rubiadipine, from which it is with difficulty freed; and the verantine is for the most part obtained in combination with oxide of tin, from which I have found it impossible to separate it.

0·3445 grm. rubiretine gave 0·8580 carbonic acid and 0·1695 water.

This gives in 100 parts—

Carbon	67·92
Hydrogen	5·46
Oxygen	26·62

0·3705 grm. verantine gave 0·9010 carbonic acid and 0·1420 water.

In 100 parts—

Carbon	66·32
Hydrogen	4·25
Oxygen	29·43

The great excess of carbon in this analysis indicates an admixture of alizarine. This proceeds from the circumstance that the method of separating verantine and alizarine, by means of acetate of copper, is not absolute. When these two substances are present together in solution, acetate of copper precipitates a portion of the alizarine together with the verantine, and the latter can afterwards only be purified by repeated solution in boiling alcohol, and deposition from the boiling solution.

The compound of verantine and oxide of tin, obtained in the process employed for purifying the rubiafine, cannot, as I mentioned before, be separated into its consti-

tuents. It dissolves in ammonia and carbonate of soda with a dark-brown colour, and is reprecipitated unchanged by acids. Even if it be dissolved in caustic soda, and an excess of sulphuretted hydrogen be passed through the solution, the precipitate afterwards produced by acids yields nothing to boiling alcohol, which proves that the verantine has not thereby been set at liberty. By treating it with boiling alcohol until all the matter soluble in alcohol is removed, then dissolving it in carbonate of soda, filtering from a small quantity of oxide of tin, then reprecipitating with acid, it is obtained in dark brown flocks, which when dry cohere into black, brittle, shining masses. Its analysis now gave the following results:—

I. 0·5930 grm., dried at 100° C., gave 0·6500 carbonic acid and 0·1800 water.

0·3410 grm., heated in a crucible until all the organic matter was destroyed, gave 0·1580 grm. peroxide of tin, equivalent to 0·1411 protoxide of tin.

II. 0·8390 grm. gave 0·9240 carbonic acid and 0·2455 water.

It contains, therefore, in 100 parts—

	I.	II.
Carbon	29·89	30·03
Hydrogen	3·37	3·25
Oxygen	25·36	
Protoxide of tin	41·38	

The formula $C_{56} H_{36} O_{36} + 7Sn O = 4C_{14} H_5 O_5 + 7Sn O + 16HO$ requires in 100 parts—

Carbon	29·76
Hydrogen	3·18
Oxygen	25·52
Protoxide of tin	41·54

The sugar obtained by the fermentation of rubian does not differ in its properties, as I mentioned before, from that derived from the action of acids on rubian. In composition too it does not materially differ from the latter. I succeeded, however, by exposing it for a considerable length of time to a temperature of 100° C., in depriving it of two atoms more of water, as will be seen by the following analysis:—

0·5540 grm. gave 0·8745 carbonic acid and 0·3055 water.

These numbers correspond with the formula $C_{12} H_{10} O_{10}$, as will be seen by the following calculation:—

	Eqs.		Calculated.	Found.
Carbon	12	72	44·44	43·84
Hydrogen	10	10	6·17	6·23
Oxygen	10	80	49·39	49·93
		162	100·00	100·00

On subjecting it, however, for some time longer to the same temperature, it became very brown, and its analysis now showed that it had absorbed oxygen.

0·5820 grm. now gave 0·8745 carbonic acid and 0·3000 water.

In 100 parts it contained therefore—

Carbon	40·97
Hydrogen	5·72
Oxygen	53·31

On redissolving the remainder in water, it gave a brown solution, but on adding to the solution hydrated oxide of lead, the brown portion was removed, while the solution became almost colourless, and on evaporation over sulphuric acid left a yellow syrup, the composition of which again corresponded with the formula $C_{12} H_{12} O_{12}$, as will be seen by the following analysis:—

0·3710 grm. gave 0·5535 carbonic acid and 0·2135 water.

In 100 parts:—

Carbon	40·68
Hydrogen	6·39
Oxygen	52·93

Rubiafine.—In my former papers on madder I have described a substance which I called rubiacine, and which I prepared partly from madder itself, and partly by the reduction of rubiacic acid with sulphuretted hydrogen. Now rubiafine cannot be distinguished by any of its properties from rubiacine. It crystallizes from its alcoholic solution in yellow glittering plates and needles, which are sometimes arranged in star-shaped or fan-shaped masses. By carefully heating it, it may be volatilized without leaving much residue, forming a yellow sublimate of small shining needles. It is but slightly soluble in boiling water. It is not decomposed by boiling nitric acid or by concentrated sulphuric acid, but merely dissolved by them. It dissolves in caustic alkalies with a reddish-purple, and in carbonated alkalies with a red colour. Its alcoholic solution gives with sugar of lead a fine crimson precipitate, with acetate of copper an orange-coloured precipitate. It dissolves in a solution of pernitrate of iron with a dark brownish-purple colour. The solution, after being boiled for some time, gives, on the addition of muriatic acid, a yellow precipitate, which is rubiacic acid. These properties, it will be seen, belong also to rubiacine. Nevertheless the composition of rubiafine is different, and as I have succeeded in again preparing a substance of the same composition as the rubiacine formerly obtained, they must be considered as distinct bodies.

I am unable to give more than one analysis of rubiafine, which yielded the following numbers:—

0·1765 grm., dried at 100° C., gave 0·4485 carbonic acid and 0·0725 water.

The relation in which rubiafine stands to rubiacic acid proves that the former contains 32 atoms of carbon. Its composition must then be as follows:—

	Eqs.		Calculated.	Found.
Carbon	32	192	69·31	69·30
Hydrogen	13	13	4·69	4·56
Oxygen	9	72	26·00	26·14
		277	100·00	100·00

If this be the true composition of rubiafine, then the latter stands in the same relation to rubian as rubianine and rubiadine, from which, if the latter also contain 32 atoms of carbon, it only differs by the elements of water. If to rubian be added 3 equivs. of water, there are given the elements of 1 equiv. of rubiafine, and 2 equivs. of sugar, for

$$\left.\begin{array}{ll}\text{2 equivs. of Sugar} \ldots & =C_{24}\,H_{24}\,O_{24} \\ \text{1 equiv. of Rubiafine} \ldots & =C_{32}\,H_{13}\,O_{9}\end{array}\right\}=\left\{\begin{array}{l}C_{56}\,H_{34}\,O_{30}=\text{1 equiv. of Rubian.} \\ H_{3}\,O_{3}=\text{3 equivs. of Water.}\end{array}\right.$$

$$C_{56}\,H_{37}\,O_{33} \qquad C_{56}\,H_{37}\,O_{33}$$

Rubiacine and Rubiacic Acid.—In my former papers on madder, I have given for rubiacic acid the formula $C_{31}\,H_{8}\,O_{16}$, and for rubiacine $C_{31}\,H_{9}\,O_{10}$. In order to ascertain the correct formula, and also to determine the relation in which they stand to rubian and rubiafine, of which they must be derivatives, I prepared and submitted to analysis a fresh quantity of these substances. For the purpose of preparing rubiacic acid, it is not necessary to take madder itself. If any dyework is at hand in which madder-dyeing is carried on, the liquor in which the goods have been immersed, together with the madder, and which is generally allowed to run to waste, may be employed. This liquid contains a considerable quantity of some substance, which by the action of pernitrate of iron may be converted into rubiacic acid. The mode of operation is as follows. To the liquid, which is brown and muddy, there is added, after the separation of the woody particles of the madder, a quantity of muriatic acid, which gives a brown flocculent precipitate, while the liquid becomes clear and colourless. This precipitate, after being collected on a calico strainer, is treated with pernitrate of iron until nothing more is dissolved. The resulting dark reddish-brown liquid is strained through calico, and acid is added to it, which produces a yellow precipitate. This is filtered, washed and dissolved in boiling carbonate of potash. The solution on cooling deposits crystals of rubiacate of potash, which are placed on a filter and washed with cold water. From a solution of the potash salt, the acid may be precipitated by muriatic or any other strong acid. Some rubiacic acid prepared in this manner was analysed with the following result:—

I. 0·3160 grm. gave 0·7090 carbonic acid and 0·1010 water.

In 100 parts it contained, therefore,—

Carbon	61·19
Hydrogen	3·55
Oxygen[1]	35·26

In my former experiments I obtained as a mean of three determinations the following numbers:—

Carbon	57·28
Hydrogen	2·47
Oxygen	40·25

I was at first unable to explain the great discrepancy here seen, as the new preparation both of the acid and the potash salt had the same appearance as usual. Before proceeding to explain how this discrepancy arose, I may state that the analysis just given corresponds with the formula $C_{64}\ H_{20}\ O_{27}$, which requires in 100 parts—

Carbon	61·93
Hydrogen	3·22
Oxygen	34·85

With the same specimen of potash salt as that employed for the preparation of the acid, I made a quantity of silver salt by dissolving the potash salt in water, and adding nitrate of silver. A brilliant cinnabar-red precipitate fell, which was filtered, washed and dried. It was not decomposed either by light or by the heat of boiling water.

0·5800 grm. of this salt, dried in the water-bath, gave 0·9800 carbonic acid and 0·1150 water.

0·3310 grm., treated in a crucible with muriatic acid, and heated until all the organic matter was destroyed, gave 0·1120 chloride of silver.

These numbers lead to the following composition:—

	Eqs.		Calculated.	Found.
Carbon	64	384	46·04	46·08
Hydrogen . . .	18	18	2·15	2·20
Oxygen	25	200	24·00	24·37
Oxide of silver .	2	232	27·81	27·35
		834	100·00	100·00

I now took a fresh quantity of waste dye liquor, and divided it into two parts. To the first part I added acid. The precipitate produced by the acid was treated with boiling alcohol, which dissolved a small part with a deep yellow colour, and after being filtered boiling hot and allowed to cool, deposited a quantity of orange-coloured powder. This powder I dissolved again in boiling alcohol, and to the boiling solution I added hydrated protoxide of tin, and filtered boiling hot. The liquid, which had become light yellow, deposited on cooling a quantity of light yellow needles. These needles had the appearance and properties of rubiafine. Their composition, however, proved them to be a distinct substance.

0·1515 grm. gave 0·3730 carbonic acid and 0·0540 water.

In 100 parts, therefore, it consisted of—

Carbon	67·14
Hydrogen	3·96
Oxygen	28·90

To the other part of the liquor I also added acid, and the precipitate, after straining the liquor through calico, and washing with water, I treated with a boiling

solution of pernitrate of iron, which dissolved a portion with a dark purplish-brown colour. Muriatic acid produced in the filtered liquid a yellow precipitate, which after filtering and washing with water, I treated with boiling alcohol. The alcohol being filtered boiling hot, deposited on cooling a yellow powder, which on being redissolved in fresh boiling alcohol, yielded a quantity of yellow shining plates and needles. These corresponded in all their properties with rubiafine and rubiacine. By treatment with pernitrate of iron they were convertible into rubiacic acid.

0·2355 grm. gave 0·5790 carbonic acid and 0·0860 water.

In 100 parts it contained, therefore,—

Carbon	67·05
Hydrogen	4·05
Oxygen	28·90

This analysis proves that the substance contained in the liquor existed in the same state before and after the treatment with pernitrate of iron, and that the latter, in the first instance at least, merely dissolved without changing it. A comparison of the composition of this substance with that of the body which I formerly called rubiacine, proves that they are identical. A specimen of rubiacine obtained on a former occasion from rubiacate of potash by means of sulphuretted hydrogen, I found to contain in 100 parts—

Carbon	67·01
Hydrogen	3·28
Oxygen	29·71

The most probable formula for this substance is $C_{32}\,H_{11}\,O_{10}$, which requires in 100 parts

Carbon	67·84
Hydrogen	3·88
Oxygen	28·28

The remainder of the substance I dissolved in boiling alcohol, and added acetate of lead. This gave a dark red precipitate, which after filtering, washing with alcohol, and drying, was found to have the following composition:—

0·5470 grm., dried at 100° C., gave 0·6630 carbonic acid and 0·0890 water.

0·4060 grm. gave 0·2800 sulphate of lead, containing 0·20602 oxide of lead.

These numbers correspond with the formula $3C_{32}\,H_{11}\,O_{10}+8PbO$, as the following calculation shows:—

	Eqs.		Calculated.	Found.
Carbon	96	576	33·05	33·05
Hydrogen	33	33	1·89	1·80
Oxygen	30	240	13·78	14·41
Oxide of lead	8	893·6	51·28	50·74
		1742·6	100·00	100·00

The precipitate produced by muriatic acid in the nitrate of iron solution, was not entirely soluble in boiling alcohol. That part left undissolved by the latter I dissolved again in nitrate of iron; I kept the solution boiling for some time, and then precipitated again with muriatic acid. The precipitate, after filtering and washing, I treated with a boiling solution of carbonate of potash, in which it dissolved. On cooling, a considerable quantity of rubiacate of potash crystallized out with its usual appearance. Some of this salt was decomposed with nitric acid, and the rubiacic acid so obtained was analysed.

II. 0·5250 grm. gave 1·1595 carbonic acid and 0·1465 water.

In 100 parts—

Carbon	60·23
Hydrogen	3·10
Oxygen	36·67

These numbers do not differ very widely from those found in the first analysis. On recrystallizing, however, the remainder of the salt from water, and analysing some acid obtained from the recrystallized salt, I obtained the following numbers:—

III. 0·4300 grm. gave 0·9135 carbonic acid and 0·1100 water.

In 100 parts—

Carbon	57·93
Hydrogen	2·84
Oxygen	39·23

With another portion of the recrystallized potash salt I prepared some silver salt, as before. 0·6260 grm. of the silver salt gave 1·0340 carbonic acid and 0·1070 water, corresponding in 100 parts to—

Carbon	45·04
Hydrogen	1·89

The mother-liquor of the salt from which No. III. was made I precipitated with muriatic acid. Part of the precipitate I dissolved again in carbonate of potash, and the acid from the crystallized potash salt showed the following composition:—

IV. 0·4010 grm. gave 0·8690 carbonic acid and 0·1160 water.

In 100 parts—

Carbon	59·10
Hydrogen	3·21
Oxygen	37·69

The remainder of the precipitate I now dissolved again in pernitrate of iron, and after keeping the solution boiling for several hours I precipitated again with muriatic acid, filtered, washed, dissolved the precipitate in carbonate of potash, filtered and precipitated again with muriatic acid. The precipitated rubiacic acid now gave on analysis the following results:—

V. 0·3925 grm. gave 0·8240 carbonic acid and 0·1030 water.

These numbers correspond with the formula $C_{32} H_9 O_{17}$, as will be seen by the following calculation :—

	Eqs.		Calculated.	Found.
Carbon	32	192	56·97	57·25
Hydrogen . . .	9	9	2·67	2·91
Oxygen	17	136	40·36	39·84
		337	100·00	100·00

This composition differs, as will be seen, very little from that found in my former experiments.

Some fresh rubiacic acid made in the same manner as that of the last analysis, was dissolved in carbonate of potash, the solution was evaporated to crystallization, the crystallized potash salt was again dissolved in boiling water, and nitrate of silver was added to the solution. The precipitate was now no longer red, but of a dull orange colour.

0·1700 grm. of this precipitate gave 0·2720 carbonic acid and 0·0370 water.

In 100 parts—

Carbon	43·63
Hydrogen	2·40

The formula $C_{32} H_8 O_{16}+AgO$ requires in 100 parts—

Carbon	43·24
Hydrogen	1·80

If the formula of the potash salt be similar to that of the silver salt, viz. $C_{32} H_8 O_{16} +KO$, it must contain in 100 parts—

Carbon	51·17
Hydrogen	2·13
Oxygen	34·12
Potash	12·58

In my former experiments I obtained as an average of three determinations—

Carbon	51·37
Hydrogen	2·41
Oxygen	33·18
Potash	13·04

It appears therefore that the four first analyses given above were made with impure acid. The analysis No. I. corresponded, as I have shown, with the formula $C_{64} H_{20} O_{27}$. Now if from this formula be deducted that of the pure acid $C_{32} H_9 O_{17}$, the difference will be $C_{32} H_{11} O_{10}$, which is the formula given above for rubiacine. It is therefore almost certain that it was an admixture of the latter substance with the acid which raised the amount of carbon and hydrogen in the four first analyses. Whether this impure acid is to be considered as a chemical compound of acid and rubiacine, or whether it contains them in a state of mechanical mixture, is a point not easily deter-

mined. That the acid of the analysis No. I. contained both substances in atomic proportions may be accidental; and the ease with which rubiacate of potash made from the impure acid yields, by mere recrystallization, a salt containing an almost pure acid, tends to prove that the two substances are merely mechanically mingled. Nevertheless it is difficult to detect the presence of rubiacine in the impure acid, which behaves towards almost all reagents in the same manner as the pure acid. The acid of the analysis No. IV., for instance, could not be distinguished from pure acid by its appearance. When treated with boiling alcohol the latter acquired a yellow colour, but on being filtered boiling hot, no rubiacine crystallized out, as would probably have been the case had the latter only been mixed with the acid. Nevertheless, on heating it cautiously between two watch-glasses, a considerable quantity of yellow shining crystals, doubtless of rubiacine, were formed on the upper glass, while the rubiacic acid of analysis No. V. gave, on being heated in the same manner, only a trace of yellow sublimate and an abundant carbonaceous residue. The potash salt of the impure acid has a more granular and less silky appearance than the salt made from pure acid, and is also of a darker red. When heated it does not detonate so strongly as the pure salt. Its solutions give the same reactions as the pure salt with all reagents except nitrate of silver, which, as I mentioned above, gives with the pure salt an orange-coloured, with the impure salt a bright cinnabar-red precipitate.

In order to ascertain whether rubiacic acid is reconvertible not only into rubiacine but also into rubiafine, I took some rubiacate of potash of the same preparation as that employed for the rubiacic acid No. I., dissolved it in boiling water, added caustic soda, and passed sulphuretted hydrogen through the solution for several hours; I then precipitated with chloride of barium, filtered, washed the precipitate, decomposed it with muriatic acid, and crystallized the residue twice from alcohol. The crystals had the appearance of rubiacine or rubiafine, and possessed considerable lustre. On analysis I obtained the following numbers:—

0·1685 grm. gave 0·4340 carbonic acid and 0·0705 water.

In 100 parts it contained therefore—

Carbon	70·24
Hydrogen	4·64
Oxygen	25·12

Though this is not exactly the composition of rubiafine as given above, still it proves that the hydrogen and oxygen are contained in it in the same proportion as in that substance; for if the formula $C_{32} H_{12\frac{1}{2}} O_{8\frac{1}{2}}$, which differs from that of rubiafine by containing $\frac{1}{2}$HO less, be calculated for 100 parts, it gives—

Carbon	70·45
Hydrogen	4·58
Oxygen	24·97

Though I have not, from want of material, been able to trace the steps of the pro-

cess with the requisite accuracy, I think I am justified in inferring from these experiments, that by the oxidizing agency of persalts of iron rubiafine is changed first into rubiacine and then into rubiacic acid, and that the latter is reconverted by the action of reducing agents, such as sulphuretted hydrogen, first into rubiacine and then into rubiafine. The presence of rubiacine, however, in the liquor which has been used for dyeing with madder, seems to prove that its direct formation from rubian is possible.

Rubiagine.—This substance belongs to the same group of bodies which includes rubianine, rubiadine and rubiafine, and bears a strong resemblance to these substances in properties and composition. It scarcely ever appears in well-defined crystals. When its alcoholic solution is evaporated spontaneously, it is obtained in the shape of small lemon-yellow spherical grains, which, when crushed and examined under a lens, are found to consist of small crystalline needles grouped round a centre. Occasionally it has an orange tinge, but this is probably due to some impurity. When heated on platinum foil, it melts to a brownish-red liquid and then burns with flame, leaving a large quantity of carbonaceous residue which burns away with difficulty. When heated in a tube, it gives a small quantity of crystalline sublimate mixed with oily drops. When slowly heated between two watch-glasses, it melts to a brownish-red mass, but gives no sublimate. It is quite insoluble in boiling water, to which it hardly communicates a tinge of colour. It is more easily soluble in boiling alcohol than rubianine or even rubiadine, and does not crystallize out on the solution cooling, but is left, on evaporation of the alcohol, in crystalline masses as just described. It is soluble in concentrated sulphuric acid with a dark reddish-brown colour; the solution, on being heated, disengages sulphurous acid and becomes black. Boiling nitric acid dissolves it with a disengagement of nitrous acid to a yellow liquid, while some oily drops rise to the surface. On the solution cooling, a quantity of light yellow crystals, possessed of much lustre, are deposited. Whether these crystals are a product of decomposition, or whether they are the substance itself in a state of purity, the impurities having been destroyed by the nitric acid, I am unable to state. The latter is the more probable view. Rubiagine is soluble in boiling acetic acid with a yellow colour, and crystallizes out again, on the solution cooling, in small needles. Ammonia turns it red, and on boiling dissolves it with some difficulty, forming a blood-red solution, which on evaporation loses its ammonia and leaves the substance behind in the shape of small yellow crystals. It dissolves more easily in caustic soda, with the same colour. It is precipitated from its alkaline solution by acids in lemon-yellow flocks. The ammoniacal solution gives very slight precipitates with the chlorides of barium and calcium, the solution remaining red with chloride of barium, and becoming crimson with chloride of calcium. It is soluble in baryta and lime-water with a blood-red colour, and is reprecipitated by a current of carbonic acid. The alcoholic solution gives, on the addition of acetate of lead, at first no precipitate, but the colour of the solution becomes dark yellow, and after some time, provided the solution be not too dilute, an orange-coloured granular

precipitate falls, which is the lead compound of rubiagine. If no deposit is formed, then the addition of water causes an orange-coloured flocculent precipitate, which, after being separated by filtration and washed with water in order to remove the acetate of lead, is found to be very little soluble in boiling alcohol, but is easily soluble in a boiling alcoholic solution of acetate of lead with a dark yellow or orange colour. Acetate of copper changes the colour of the alcoholic solution from light yellow to brownish-yellow, and after some time an orange-coloured precipitate is formed. When rubiagine is treated with a boiling solution of perchloride of iron, the solution acquires a darker colour, but does not assume the deep brownish-purple characteristic of solutions of rubiafine and rubiacine in that menstruum. The liquid being filtered boiling hot, deposits on cooling a quantity of yellow shining scales, but the addition of muriatic acid produces no further precipitate. These scales dissolve easily in boiling alcohol, and the solution on cooling and standing deposits a number of small yellow grains and nodules consisting of crystalline needles, which are apparently nothing but rubiagine itself, for they are not capable of sublimation, and their alcoholic solution is not precipitated by acetate of lead. The greatest part of the rubiagine is left undissolved by the perchloride of iron in the shape of a yellowish-brown powder, which does not dissolve on treating it with an additional quantity of the iron salt. Boiling muriatic acid changes the colour of this powder to yellow, and it has then all the properties of rubiagine. Rubiagine is therefore not changed into rubiacic acid by the action of persalts of iron. Notwithstanding the great resemblance which rubiagine bears to the other bodies belonging to the same series, its reactions prove it to be a distinct substance. It is distinguished from rubianine by its insolubility in water; from rubiadine, for which it might most easily be mistaken, by its being incapable of sublimation; and from rubiafine by its not being convertible into rubiacic acid. Its behaviour towards acetate of lead, which is different from that of all the other three substances, also serves to characterize it.

The analysis of rubiagine gave the following results:—

0·3800 grm., prepared directly from madder, gave 0·9490 carbonic acid and 0·1760 water.

In 100 parts it contained therefore—

Carbon	68·10
Hydrogen	5·14
Oxygen	26·76

There are two formulæ with which this analysis corresponds, and both of which explain the formation of the substance equally well, viz. $C_{32}H_{14}O_{10}$ and $C_{44}H_{17}O_{13}$. These formulæ require respectively in 100 parts—

	$C_{32}H_{14}O_{10}$.	$C_{44}H_{17}O_{13}$.
Carbon	67·13	68·57
Hydrogen	4·89	4·41
Oxygen	27·98	27·02

I am unwilling to draw any inference from the greater or less correspondence of either of these calculations with the experimental result, because I am not convinced of the absolute purity of the specimen employed for analysis.

The lead compound of rubiagine was prepared by adding a small quantity of an alcoholic solution of sugar of lead to a concentrated alcoholic solution of the substance, taking care not to employ an excess of the precipitant. The orange-coloured precipitate was collected on a filter, washed with alcohol, dried and submitted to analysis.

0·4610 grm., dried in the water-bath, gave 0·5290 carbonic acid and 0·1110 water.

0·2010 grm. gave 0·1460 sulphate of lead, containing 0·10742 oxide of lead.

In 100 parts it contained therefore—

Carbon	31·29
Hydrogen	2·67
Oxygen	12·60
Oxide of lead	53·44

These numbers do not decide between the two formulæ, since they correspond equally well with $C_{32} H_{14} O_{10}+3PbO$ and $C_{44} H_{17} O_{13}+4PbO$, as the following calculation shows:—

	Eqs.			Eqs.		
Carbon	32	192	30·91	44	264	31·73
Hydrogen	14	14	2·25	17	17	2·04
Oxygen	10	80	12·89	13	104	12·53
Oxide of lead	3	335·1	53·95	4	446·8	53·70
		621·1	100·00		831·8	100·00

If $C_{32} H_{14} O_{10}$ be the true formula of rubiagine, then it is formed by rubian taking up 4 equivs. of water and splitting up into 1 equiv. of rubiagine and 2 equivs. of sugar, as seen by the following equation:—

$$\left.\begin{array}{ll}\text{2 equivs. of Sugar} & =C_{24} H_{24} O_{24} \\ \text{1 equiv. of Rubiagine} & =C_{32} H_{14} O_{10}\end{array}\right\} = \left\{\begin{array}{l}C_{56} H_{34} O_{30}=1 \text{ equiv. of Rubian.} \\ H_4 O_4=4 \text{ equivs. of Water.}\end{array}\right.$$

$$C_{56} H_{38} O_{34} \qquad C_{56} H_{38} O_{34}$$

If, on the other hand, $C_{44} H_{17} O_{13}$ be the correct formula, then 1 equiv. of rubian loses 5 equivs. of water, and splits up into 1 equiv. of rubiagine and 1 equiv. of sugar, for

$$C_{44} H_{17} O_{13}+C_{12} H_{12} O_{12}+5HO=C_{56} H_{34} O_{30}.$$

Rubiadipine.—This substance is a characteristic product of the fermentation of rubian. I have never subjected rubian to the action of ferment, under ordinary circumstances, without being able to detect it among the bodies formed. In its appearance and general properties it resembles rubiretine. It differs from the latter in always remaining soft and viscid, and never becoming hard and brittle, however long it may be heated. It is similar in appearance to a semifluid fat tinged with

colouring matter. Its colour is yellowish-brown. When heated on platinum foil it melts to a brown liquid and then burns with a bright flame, leaving a carbonaceous residue. When heated in a tube it evolves acrid fumes, similar to those produced by fat when exposed to destructive distillation. It is not much affected by boiling nitric acid, but concentrated sulphuric acid chars it when heated. When thrown into boiling water rubiadipine melts, forming oily drops, which rise to the surface. It is soluble in caustic alkalies with a blood-red colour, but the solutions do not froth when boiled like solutions of soap. The ammoniacal solution gives only a slight precipitate with chloride of barium. On adding to the alcoholic solution a small quantity of acetate of lead, a pale reddish-brown precipitate is formed, which is the lead compound. This precipitate is insoluble in boiling alcohol, but dissolves entirely when an excess of acetate of lead is added to the boiling liquid, forming a dark brownish-red solution. From this solution it is again precipitated by water, and after filtering and washing is found to be again insoluble in boiling alcohol. In its behaviour to sugar of lead it therefore resembles rubiagine. The alcoholic solution gives no precipitate on the addition of acetate of copper. The substance itself cannot be obtained in a state fit for analysis, I therefore confined myself to the examination of the lead compound formed in the manner just described. The quantity of the substance obtained was, nevertheless, so small that I had only sufficient for one analysis at my disposal.

0·2020 grm., dried at 100° C., gave 0·3770 carbonic acid and 0·1260 water.

0·1150 grm. gave 0·0490 sulphate of lead, containing 0·03605 oxide of lead.

These numbers lead to the formula $C_{30}H_{24}O_5+PbO$, as the following calculation shows:—

	Eqs.		Calculated.	Found.
Carbon	30	180	50·60	50·89
Hydrogen . . .	24	24	6·74	6·93
Oxygen	5	40	11·26	10·83
Oxide of lead . .	1	111·7	31·40	31·35
		355·7	100·00	100·00

If this formula represents the true composition of rubiadipine, I confess I am unable to explain its formation from rubian. The great excess of hydrogen contained in it, shows that some substance must be formed simultaneously containing a large proportion of oxygen, but which has hitherto escaped detection.

The experiments above detailed prove that the decomposition which rubian undergoes by fermentation, does not differ in its general nature from that which results from the action of acids or alkalies. The decomposing effect of the ferment extends like that of these agents to three portions of rubian. The first portion of rubian loses water and gives rise to the formation of alizarine. The second loses water and produces rubiretine and verantine in equivalent proportions. The third takes up water and gives sugar and rubiafine, or it takes up 1 equiv. more of water, and yields sugar

and rubiagine. The rubianine produced by acids, and the rubiadine formed by alkalies, give place, when ferment is the decomposing agent, to rubiafine and rubiagine. The rubiadipine, in consequence of the uncertainty of its nature and origin, and the minute quantity in which it is formed, I leave out of consideration. The relative proportion in which the various products of decomposition are formed, seems to be about the same in the case of ferment as when acids or alkalies are employed. Alizarine is formed in the smallest quantity; of rubiretine and verantine a little more is produced, but the sugar and the rubiafine and rubiagine exceed all the others in amount.

It appears further from these experiments, that the ordinary process of dyeing with madder is, in fact, mainly a process of fermentation, that it consists essentially of a re-arrangement of the elements of rubian induced by the action of ferment, whereby a formation of colouring matter takes place, and that the precautions necessary to be taken in regard to the regulation of the temperature in madder dyeing have reference principally to the progress of the fermentation. The extraordinary rapidity with which this process of fermentation is completed, is the only circumstance which distinguishes it from other processes of a similar nature.

A very interesting question now arises, suggested by the analogy which must be presumed to exist between this and other processes of fermentation, viz. By what means is the action of the ferment on rubian either destroyed, retarded or promoted; and do not any means exist of so modifying the action as to lead to the formation of particular substances in preference to others? With the view of throwing some light on this question, I instituted a series of experiments, which I shall now proceed to describe.

Having taken a solution of rubian and mixed it with a quantity of ferment sufficiently large to effect its decomposition under ordinary circumstances, I raised the temperature of the mixture to the boiling point and kept it boiling for a short time. After the mixture had stood for some days exposed to a moderate temperature, I found that the rubian had not undergone the least change. The liquid remained yellow and bitter, and after filtration and evaporation left a residue of rubian with its usual appearance and properties.

I took in the next place a quantity of ferment, dried it slowly at a moderate temperature, reduced it to a fine powder and mixed it with a solution of rubian. After the mixture had stood for some days, the rubian was entirely decomposed. The filtered liquid left on evaporation a quantity of sugar, and the residual mass on the filter, which was interspersed with numerous small yellow crystals, was treated with boiling alcohol, which, after being filtered boiling hot and allowed to cool, deposited a large quantity of rubiafine in crystals. The alcoholic liquid, on being examined in the usual manner, was found to contain besides rubiafine, alizarine, rubiagine, rubiretine and verantine, the two latter being rather less in amount than usual.

After drying the ferment at a moderate temperature, then heating it for some hours

in a water-bath, before adding it to a solution of rubian, no apparent change took place for some time, but gradually the rubian began to disappear, and after two months was entirely decomposed. The filtered liquid left on evaporation a quantity of sugar, and the residue on the filter, on being treated with alcohol, yielded a solution which contained rubiafine, and a pretty large quantity of rubiretine and verantine, but no alizarine.

To a solution of rubian I now added a sufficient quantity of ferment to effect its decomposition when used alone, and then a small quantity of sulphuric acid. The mixture remained apparently unchanged for some time. The liquid was filtered, the acid was neutralized with carbonate of lead, the liquid was then again filtered, and left on evaporation a yellowish-red, bitter, glutinous substance, which was apparently a mixture of rubian and sugar. The mass left on the filter was treated with boiling alcohol, which was then found to contain a trace of alizarine, a little rubiagine, and a large quantity of rubiretine and verantine.

The same experiment was performed, substituting for the sulphuric acid a minute quantity of carbonate of soda. This had the effect of completely dissolving the ferment, forming a solution to which the rubian imparted a red colour. After standing for some time, the rubian was entirely decomposed, for on neutralizing the soda with sulphuric acid, a yellow precipitate fell, while the liquid became colourless. The latter being filtered, the acid was neutralized with carbonate of lead, and the liquid being again filtered left on evaporation a quantity of sugar mixed with sulphate of soda. The mixture of ferment with the products of decomposition yielded to boiling alcohol a large amount of rubiafine, rather more than the average quantity of alizarine, and a moderate quantity of rubiretine and verantine, but no rubiagine. I repeated this experiment, using caustic soda instead of carbonate of soda; but having employed rather too large a quantity of alkali, I found that the action of the ferment was very much retarded, so much so that after standing for some days there was still a quantity of undecomposed rubian present; and among the products of decomposition formed there was little or no alizarine, rubiafine or rubiagine, but a large quantity of rubiretine and verantine. The addition of lime water produced a similar effect to that of carbonate of soda, though of not quite so marked a character.

Having mixed a solution of rubian with ferment in the usual manner, I added a small quantity of sugar of lead. The ferment, which had been previously diffused through the liquid, was immediately precipitated by the lead salt in brown flocks, leaving a clear yellow solution floating above it. After standing however for some days the colour of the solution became gradually paler, and at length almost disappeared. After filtration and evaporation it left a small quantity of sugar. The mass left on the filter was purple, and contained oxide of lead. It was treated with cold sulphuric acid, which turned it red. The acid liquid after filtration was neutralized with carbonate of lead, and again filtered, when it appeared colourless, and produced no precipitate with basic acetate of lead, a proof that it contained no rubian. The mass

which had been treated with cold sulphuric acid, was then treated with boiling alcohol, which on being filtered and allowed to cool deposited some crystallized rubiafine, and was found to contain a trace of alizarine and a large quantity of rubiretine and verantine. It appears therefore that even sugar of lead, which produces an insoluble compound with the ferment, is incapable of arresting the action of the latter on rubian.

The same experiment was made, using corrosive sublimate instead of sugar of lead. The action of the ferment was in this case considerably retarded, for the yellow colour of the liquid did not entirely disappear, even after several days. The filtered liquid left on evaporation some corrosive sublimate with a little rubian, but no sugar. The mass on the filter was treated with boiling alcohol, which was then found to contain no alizarine, rubiafine or rubiagine, but a considerable quantity of rubiretine and verantine.

Arsenious acid had a very similar effect to sugar of lead and corrosive sublimate, that is to say, it retarded the action of the ferment, prevented the formation of alizarine, and promoted that of rubiretine and verantine.

The addition of a large quantity of alcohol to a mixture of rubian and ferment had likewise the effect of retarding, though not of altogether preventing the fermentation. A great part of the rubian remained undecomposed, while the remainder had given rise to the formation of a small quantity of alizarine and a large quantity of rubiretine and verantine.

A solution of rubian having been mixed with ferment, I poured the mixture into a bottle, and then added a quantity of oil of turpentine, after which the whole was well shaken up and left to stand. The decomposition of the rubian was much retarded, and took several days for its completion. The oil of turpentine floating on the surface was removed. The liquid underneath was colourless. After filtration and evaporation it left a quantity of sugar. The remaining products of decomposition were rubiafine, a trace of alizarine, and a large quantity of rubiretine and verantine, but no rubiagine.

From these experiments, which seem to me sufficiently decisive to require no further confirmation, I draw the following inferences:—

1. There exist no means, short of the complete destruction of the ferment, capable of arresting its action on rubian, except exposing it while in a moist state to the temperature of boiling water. Even when exposed to that temperature, after having been previously dried, its fermenting power is not entirely lost, but merely weakened.

2. By the addition of various substances, usually classed as antiseptic, such as sulphuric acid, arsenious acid, sugar of lead, corrosive sublimate, alcohol and oil of turpentine, during the process of fermentation the action of the ferment is not destroyed; it is merely retarded and modified.

3. The more the action of the ferment on rubian is retarded, the more rubiretine and verantine, and the less alizarine are formed, so much so that in some cases the

alizarine disappears entirely from among the products of decomposition, which then consist almost solely of rubiretine and verantine. The formation of rubiafine and rubiagine is promoted when the action of the ferment is moderately retarded, but diminishes again, or entirely ceases when the retardation is very great. Of the two, the rubiagine is the first to disappear when any retardation takes place.

4. By the addition of small quantities of alkalies during the process of fermentation, the action is, as regards its duration, if not promoted, at all events not retarded; and as regards the relative quantities of the various substances produced, the amount of alizarine is thereby decidedly increased, while that of the rubiretine and verantine is diminished.

These experiments also confirm the view derived from analytical results, that there is a mutual relation and interdependence on the one hand between rubiretine and verantine, and on the other between rubiafine and rubiagine and the sugar, whereas alizarine occupies an independent position; for rubiretine is never found among the products of decomposition by fermentation without an accompaniment of verantine, though both may be present to the exclusion of all other products; and the formation of sugar always indicates that of rubiafine or rubiagine, whereas all these substances may be present without a trace of alizarine being at the same time produced.

The deleterious effect resulting from the presence of acids in the dye-bath during the process of madder dyeing hereby finds an additional explanation. Not only do acids act injuriously by seizing hold of the mordants with which the colouring matter ought to combine, not only do they allow the constituents of the root which are injurious in the process to have full scope by depriving them of the alkalies or alkaline earths with which they would otherwise unite, but they also retard the peculiar process by which the alizarine is formed, and even lead to the formation of deleterious substances at the expense of the colouring matter itself.

In order to place in a more striking light the influence exerted respectively by acids and alkalies during the process of the fermentation of rubian, I will here give the results of an experiment to ascertain quantitatively the amount of the various products of decomposition formed on the addition of either during the process. For this purpose I took 5·59 grms. rubian, dissolved it in water, added to the solution a quantity of ferment, and mixed both together very well. I then divided the mixture into two equal parts, and added to one half a small quantity of carbonate of soda, to the other a little sulphuric acid. Both were allowed to ferment for a length of time, and the portion insoluble in water was treated in both cases with boiling alcohol. The alcohol deposited on being filtered boiling hot a quantity of rubiafine, which was collected on a filter and weighed. To the alcoholic liquid I added acetate of alumina. The precipitate thereby occasioned was separated by filtration, washed with alcohol and decomposed with boiling muriatic acid. The red flocks thus obtained were collected on a filter, washed, dried and weighed. They consisted of alizarine, verantine and rubiafine. After being weighed, they were treated with boiling dilute nitric

acid, which destroyed the alizarine, leaving the two other substances behind in the shape of a yellow powder, which was filtered, washed, dried and weighed. By deducting its weight from the total weight of the three, the weight of the alizarine was ascertained. The liquid filtered from the alumina precipitate deposited on the addition of sulphuric acid and water a yellow powder, which was collected on a filter, washed, dried and weighed. It was then treated with a little cold alcohol. This left undissolved a quantity of rubiagine, which was again collected on a filter, dried and weighed. The alcohol left on evaporation a dark brown mass, the fatty nature of which showed that it contained rubiadipine in addition to rubiretine. Now I obtained from that half of the solution to which carbonate of soda had been added, the following quantities of these various substances:—0·323 grm. alizarine, 0·220 grm. rubiafine, 0·167 grm. of the mixture of rubiafine and verantine, 0·250 grm. rubiagine, and 0·170 grm. of the mixture of rubiretine and rubiadipine. From the other half, to which sulphuric acid had been added, I obtained the following quantities of the same bodies:—0·2030 grm. alizarine, 0·340 grm. rubiafine, 0·157 grm. of the mixture of rubiafine and verantine, 0·310 grm. rubiagine, and 0·230 grm. of the mixture of rubiretine and rubiadipine. In both cases a quantity of rubian remained undecomposed, but the quantity actually decomposed was capable of being approximately determined from the quantities of products of decomposition formed. Assuming the formula of rubiafine to be $C_{32}H_{13}O_{9}$, that of rubiagine $C_{32}H_{14}O_{10}$, assuming further that the mixture of rubiafine and verantine consisted entirely of the latter substance, by which no great error is committed (since the quantities of rubiafine and verantine which one atom of rubian is capable of yielding are to one another as 277 to 258), and leaving out of consideration the trifling quantity of rubiadipine formed (the rubiretine being given by the amount of verantine), then the quantities of the various substances obtained correspond respectively to the following quantities of rubian:—

		Rubian.		Rubian.
Alizarine .	0·323 corresponds to	0·407	0·203 corresponds to	0·255
Rubiafine .	0·220 corresponds to	0·484	0·340 corresponds to	0·748
Rubiagine .	0·250 corresponds to	0·533	0·310 corresponds to	0·661
Verantine .	0·167 corresponds to	0·394	0·157 corresponds to	0·371
		1·818		2·135

Hence it appears that though the amount of rubian which actually underwent decomposition was less when carbonate of soda was added than when sulphuric acid was employed in addition to the ferment, still the amount of the alizarine produced was greater in the former than the latter case, while the amount of almost all the other substances was proportionally less. In using carbonate of soda, the quantity of alizarine formed amounted to 17·7 per cent., in using sulphuric acid it amounted to 9·5 per cent. of the rubian decomposed. The quantity which should be formed, if the rubian were entirely converted into alizarine, is 79·3 per cent.

In the first part of this paper I have pointed out the possibility which exists in theory of converting rubian entirely into alizarine, to the exclusion of every other product of decomposition; and though I cannot say that I have approached much nearer to its practical realization, still if, as the experiments just described prove, the fermentative process whereby alizarine is formed is of so plastic a nature as to be capable of yielding under different circumstances, sometimes more, sometimes less, occasionally none, of the latter substance, then the prospect of our being able so to modify it as to lead to the formation of a much larger quantity of alizarine from a given quantity of rubian than is at present possible, becomes less distant.

The experiments which I shall now describe were made in order to ascertain the effect produced on rubian by other fermentative substances besides that contained in madder itself.

I first took a solution of rubian and mixed it well with a solution of albumen made from white of egg. The mixture was allowed to stand in a moderately warm place for two months, but though the albumen had entered into a state of putrefaction, the rubian was still for the most part undecomposed, as was evident from the taste and colour of the liquid. At the bottom of the vessel there was found a small quantity of a dark reddish-brown deposit, which was collected on a filter. On treating this deposit with boiling alcohol, the latter acquired a red colour, and left on evaporation a small quantity of a yellow bitter substance, soluble in water, which only differed from rubian in being somewhat viscid. The remainder of the dark brown deposit I treated with a mixture of muriatic acid and alcohol, when it immediately became almost white, while the substance which had coloured it dissolved in the alcohol with a yellow colour. The alcohol was filtered from the insoluble part, which was probably merely modified albumen, and evaporated to dryness, when it left a reddish-yellow, uncrystalline residue. This residue was impure alizarine, for it gave on being heated in a tube a crystalline sublimate, it dissolved in caustic alkali with a violet colour, and its alcoholic solution became purple on the addition of acetate of copper. In the reddish-brown deposit it was doubtless contained in combination with some base, perhaps lime. The greatest part of the rubian remained in solution mixed with the albumen. The solution was slowly evaporated to dryness, when it left a red transparent substance like gum or varnish, which was treated with boiling alcohol. The alcohol acquired a reddish-yellow colour, and left on evaporation a yellowish-red, deliquescent, bitter residue, consisting apparently of rubian mixed with some nitrogenous substance. It was entirely soluble in water with a reddish colour. This solution gave with acetate of lead a copious brown precipitate, the supernatant liquid being almost colourless, but with acids and alkalies it gave the usual reactions of a solution of rubian. The greater part of the gum-like substance was of course insoluble in alcohol. On being treated with a mixture of sulphuric acid and alcohol in the cold, an additional quantity of yellow liquid was obtained, which after filtration, neutralization with carbonate of lead and evapora-

tion, left a reddish, deliquescent residue similar to the last. It appears, therefore, that the action of albumen on rubian is but slight.

In order to ascertain what effect caseine has on rubian, I mixed a solution of the latter with a quantity of milk, and allowed the mixture to stand in a warm place for several days, until the smell indicated that the caseine had entered into a state of putrefaction. The liquid, which was still yellow and bitter, was filtered from the caseine, which had separated in yellow flocculent masses, and basic acetate of lead was added, which produced a red precipitate. This precipitate, after filtering and washing, was decomposed with cold sulphuric acid; the excess of sulphuric acid was removed with carbonate of lead; and after passing sulphuretted hydrogen through the filtered liquid to precipitate a little lead contained in the solution, it was again filtered and evaporated, when it left a quantity of rubian possessing the usual properties of that substance, with the exception of its being somewhat viscid. The caseine on the filter after being washed with water was treated with boiling alcohol, which deprived it of its yellow colour, and rendered it white. The filtered liquid, which was yellow, gave no precipitate either with acetate of alumina or sugar of lead, proving the absence of alizarine and the usual products of decomposition of rubian. On evaporation it left a residue of rubian mixed with fat, the whole, with the exception of the fat, being soluble in water.

A similar negative result was obtained with gelatine and yeast. A solution of rubian being mixed with a solution of glue, the mixture after standing for several weeks began to emit a putrid smell, and acquired a reddish tinge, but remained bitter, and deposited nothing insoluble. On adding yeast to a solution of rubian, and allowing to stand until putrefaction commenced, no apparent change took place in the solution, which retained its yellow colour and bitter taste.

The action of emulsine on rubian was very different. The emulsine employed was prepared in the usual manner, by treating sweet almonds, after being pounded and pressed between paper to deprive them of their oil, with water, separating the milky liquid from the residue by straining, allowing it to stand until the fat had collected on the surface, and then precipitating the emulsine from the clear liquid underneath, by means of alcohol. Some emulsine prepared in this manner was placed in a solution of rubian, and well mixed up with it. The mixture was allowed to stand in a warm place for some days. By degrees the liquid became almost colourless, while the emulsine acquired a yellow colour. It was filtered and evaporated, when it left a quantity of emulsine. The yellow mass on the filter was treated with boiling alcohol, which became yellow, while the residue lost almost the whole of its colour. The alcoholic liquid was filtered and spontaneously evaporated, when it left a yellowish-red mass, among which crystals of alizarine were discernible. This mass was treated with cold water, in which a part dissolved with a yellow colour. The liquid after filtration and evaporation left a quantity of unchanged rubian. The part insoluble in water was dissolved again in alcohol, and to the solution was added

acetate of alumina, which produced a red precipitate. This precipitate, after filtration and washing with alcohol, was decomposed with muriatic acid, and the yellow flocks left by the acid were dissolved in alcohol, on the spontaneous evaporation of which there was left a mass of pure alizarine in well-defined yellow crystals. The liquid filtered from the alumina precipitate was evaporated to dryness, and the residue left was treated with water and muriatic acid, when a brown mass was obtained, which after filtering and washing was treated with cold alcohol. The alcohol left undissolved a brown powder, consisting probably of verantine, and after filtration and evaporation left a brown resinous substance, easily fusible when thrown into boiling water, and consisting doubtless of rubiretine. The quantity of alizarine formed seemed to be much larger in proportion to the rubian employed, and to the quantity of other substances formed, than in the case of the ferment of madder. Nevertheless, I must state, that on repeating the experiment I was unable to attain the same result, the alizarine being formed in much smaller proportion and giving place to other products of decomposition.

Lastly, I resolved to examine what effect, if any, would be produced on rubian by seme fermentative substance derived like that of madder from a vegetable root. For this purpose I chose the albuminous substance described by BRACONNOT as existing in the root of the *Helianthus tuberosus**. I selected this substance, because it has, like the ferment of madder, the property of causing a solution of sugar to undergo an acetous fermentation. It was prepared in the following manner. The tubers were cut into slices, then pounded in a mortar with a little water until the lumps had disappeared. The mass was then placed on a piece of calico, and the liquor expressed with the hands. The muddy liquid which ran through was then mixed with a considerable quantity of alcohol. This produced a gray precipitate, which was collected on a filter and well washed with cold alcohol. Some of this precipitate was then added to a solution of rubian, and the mixture was allowed to stand in a moderately warm place until it began to disengage a putrid smell. The liquid was then filtered, and evaporated to dryness. The residue was treated with boiling alcohol, which dissolved a part with a yellow colour, leaving undissolved a quantity of brown flocks, consisting probably of some of the ferment. The alcohol on evaporation left a residue, consisting of rubian with its usual appearance and properties, and apparently free from sugar. The substance left on the filter had acquired a brownish-yellow colour, which was not removable by washing with water. On being treated with boiling alcohol, it lost its yellow colour, which was now transferred to the alcohol. The alcohol after filtration and evaporation left a dark brown substance in the shape of a pellicle, which yielded nothing to boiling water. On being treated with cold alcohol, a brown powder resembling verantine was left undissolved, while the filtered liquid gave on evaporation a residue of a resinous nature, which melted when thrown into boiling water and became brittle again when cold, and consisted doubtless of rubire-

* Ann. de Chem. et de Phys. xxv. 358.

tine. The effect produced by this substance on rubian, therefore, though it does not equal in energy that of the madder ferment itself, or even of emulsine, exceeds that of albumen or caseine. As regards the substances produced by it, its action resembles that of madder ferment when retarded by the addition of antiseptic substances, and it confirms the law which I have deduced from previous experiments, viz. that the more slowly rubian is decomposed, the more rubiretine and verantine are produced.

It appears from these experiments, that none of the common and well-known fermentative substances, with the exception of emulsine, are capable of effecting in any considerable degree the decomposition of rubian, and that none of them, with that single exception, can be employed as a substitute for the ferment contained in madder itself, which produces an effect on rubian altogether *sui generis*. This circumstance alone, apart from all other considerations, would entitle the ferment of madder to be considered as an entirely distinct and peculiar substance, on which it will therefore be necessary to bestow a distinct name. For this purpose I venture to suggest the name of *Erythrozym* (from ἐρυθρὸς *red*, and ζύμη *ferment*) as most appropriately indicating its chief characteristic, and I shall now proceed to give a short account of its properties and composition.

When prepared in the manner above described by precipitation with alcohol, erythrozym is obtained as a chocolate-coloured granular mass. When dried it coheres into hard lumps, which are almost black, and are with difficulty reduced to powder. When the dry substance is heated on platinum foil it emits a smell somewhere between that of burning peat and burning horn, and then burns without much flame, leaving a considerable quantity of residue, which, on being further heated, is soon converted into a grayish-white ash, consisting almost entirely of carbonate of lime. If erythrozym be well mixed while in a moist state with water, a reddish-brown muddy liquid is formed, having all the appearance of a solution. It is, however, no solution; the erythrozym is merely suspended in the liquid, for on filtering through paper a clear liquid passes through, while a mucilaginous substance remains on the filter. The latter, on being mixed with a solution of rubian, exerts the usual decomposing effect on that substance, while the liquid, when tried in the same way, is found to be entirely without effect. Hence it follows that erythrozym, after having once been precipitated from its watery solution, even by alcohol, cannot again be dissolved in water. The liquid obtained by treating erythrozym with water and filtering, contains a small quantity of a substance, which, from its reactions, I conclude to be pectic acid, or some body nearly allied to it. In fact, the method of preparing erythrozym implies that all substances contained in the watery extract of madder, insoluble in alcohol, must be found mixed with it; but since the erythrozym itself by precipitation with alcohol becomes insoluble in water, these substances may afterwards be easily removed by treating with water. If the watery liquid in which the erythrozym is contained in a state of suspension be boiled, a sort of coagulation takes place, and the erythrozym separates in the shape of dirty red flocks, while the liquid retains a reddish colour. The same effect is produced by adding alcohol or salts, such as

common salt or sal-ammoniac, the substance separating in dark reddish-brown flocks, with a clear yellowish liquid floating above them. That erythrozym is not an uncombined substance, but a compound of an organic substance with lime, is proved by its behaviour towards acids. If it be treated with any acid, even acetic acid, its colour changes from reddish-brown to yellowish-brown, and the filtered liquid is found to contain a considerable quantity of lime. The yellowish-brown flocks left on the filter, after all the excess of acid has been removed, do not again form with water a mucilaginous liquid like the original substance; and even an addition of lime water, though it restores the original chocolate colour, does not reproduce that peculiar condition of suspensibility in water characteristic of it in its original state. The brown substance into which erythrozym is changed by the action of acids is soluble in caustic alkalies, forming pale purple turbid liquids. The ammoniacal solution gives reddish-brown flocculent precipitates with most earthy and metallic salts. When the solution in caustic soda is boiled, a disengagement of ammonia takes place. If erythrozym be treated with boiling nitric acid, it is dissolved and decomposed with a disengagement of nitrous acid. A small quantity of a white flocculent substance remains behind. Concentrated sulphuric acid chars it on heating.

If erythrozym be mixed with water, and the mixture be allowed to stand for a length of time in a warm place, signs of a more active process of fermentation begin to show themselves, especially in summer weather; bubbles of gas are given off, and a peculiar smell is emitted, which, though disagreeable, cannot exactly be called putrid. During this process, which is evidently one of putrefaction in the stricter sense, the erythrozym loses its sliminess, and is converted into a red flocculent mass, which may easily be separated by filtration from the liquid. The latter is clear, colourless and quite neutral. After erythrozym has passed through this second stage of decomposition, its power of decomposing rubian is found to have lost much of its intensity. It is during the first period of its decomposition, when no apparent change is taking place, that this power is most energetically exerted. During the second, or more strictly putrefactive stage, it acquires, however, the property of decomposing sugar. If erythrozym be mixed with a solution of cane sugar, and the mixture be allowed to stand for a considerable time until gas begins to be disengaged, the solution acquires by degrees a decided acid reaction. What the acid is which is thereby formed, I have not yet ascertained.

The erythrozym which I submitted to analysis, was prepared by precipitating it from an extract of madder with tepid water, by means of alcohol, collecting it on a filter, then treating it repeatedly with boiling alcohol until all matter soluble therein was removed, and then washing it on a filter with cold water until the percolating liquid no longer gave a precipitate with sugar of lead, after which it was rapidly dried in the water-bath. It follows from the analyses which I have made, that the amount of lime which it contains is tolerably constant, and that it must consequently be considered as a definite compound of an organic substance with lime.

I. 0·6220 grm., dried at 100° C. and burnt with oxide of copper and chlorate of potash, gave 0·8670 carbonic acid and 0·2365 water.

0·7770 grm. gave 0·4035 chloride of platinum and ammonium.

0·9630 grm. gave on being incinerated 0·2350 carbonate of lime.

II. 0·4885 grm. of another preparation gave 0·6865 carbonic acid and 0·2060 water.

0·1540 grm. gave on being incinerated 0·0370 carbonate of lime, containing 0·02076 lime =13·48 per cent. After being treated with sulphuric acid and again heated, it weighed 0·0500 grm., which, estimated as sulphate of lime, is equivalent to 0·02062 lime =13·39 per cent.

From these numbers may be deduced the formula $C_{56}H_{34}N_2O_{40}+4CaO$, as the following calculation shows:—

	Eqs.		Calculated.	I.	II.
Carbon	56	336	40·48	40·93	41·20
Hydrogen . . .	34	34	4·09	4·22	4·68
Nitrogen . . .	2	28	3·37	3·26	
Oxygen	40	320	38·57	37·90	
Lime	4	112	13·49	13·69	13·48
		830	100·00	100·00	

If this be the true composition of erythrozym, it stands in a very interesting relation to that of rubian. If to 1 equiv. of rubian be added the elements of 2 equivs. of nitric acid, the sum will represent the composition of the organic substance contained in the erythrozym in combination with lime, $C_{56}H_{34}O_{30}+2NO_5=C_{56}H_{34}N_2O_{40}$.

I shall now give the results of an analysis of erythrozym which had been employed for the decomposition of a quantity of rubian, and then treated successively with cold water and boiling alcohol until all the products of decomposition were entirely removed.

0·5330 grm., dried at 100° C. and burnt with oxide of copper and chlorate of potash, gave 0·8325 carbonic acid and 0·2220 water.

0·2780 grm. gave 0·1835 chloride of platinum and ammonium.

0·0645 grm. gave on being incinerated 0·0130 carbonate of lime, containing 0·00729 lime.

These numbers lead to the following composition:—

	Eqs.		Calculated.	Found.
Carbon	52	312	44·82	44·99
Hydrogen . . .	32	32	4·59	4·62
Nitrogen . . .	2	28	4·02	4·14
Oxygen	30	240	34·51	34·95
Lime	3	84	12·06	11·30
		696	100·00	100·00

If this composition be compared with that of the substance in its undecomposed state, the exact nature of the change which it undergoes during the process of fermentation will be apparent. It appears that the change consisted in this case in the loss of 4 atoms of carbonic acid, 2 atoms of water and 1 of lime, for

$$C_{52} H_{32} N_2 O_{30} + 3CaO + 4CO_2 + 2HO + CaO = C_{56} H_{34} N_2 O_{40} + 4CaO.$$

It appears also that no loss had been sustained in the nitrogen. The atom of lime lost was probably abstracted by the carbonic acid disengaged at the same time.

In order to obtain, if possible, the organic substance, which is contained in the erythrozym in combination with lime, and to which the name of erythrozym ought perhaps to be restricted, in a free state, I prepared an extract of madder with tepid water, and added to it tartaric acid. A brown precipitate was produced, which was collected on a paper filter, washed with water in order to remove the excess of acid, then treated with boiling alcohol, until everything soluble in that menstruum was removed, and lastly, washed with cold water, in order to separate any pectic acid which might be present; after which it was dried. Its analysis showed, however, that the acid had removed only half of the lime, and that the substance itself must, during the process of purification, have undergone decomposition.

0·5695 grm., burnt with oxide of copper and chlorate of potash, gave 0·9585 carbonic acid and 0·2600 water.

0·5940 grm. gave on being incinerated 0·0900 carbonate of lime, containing 0·0505 lime =8·50 per cent.

0·2835 grm. gave on being incinerated, and then treated with sulphuric acid, 0·0560 sulphate of lime, containing 0·02310 lime =8·14 per cent. The average per-centage of lime was therefore 8·32.

The nitrogen was not determined; but presuming no loss to have taken place in that constituent, then these numbers correspond to the following composition:—

	Eqs.		Calculated.	Found.
Carbon	52	312	48·00	47·68
Hydrogen . . .	30	30	4·61	5·07
Nitrogen . . .	2	28	4·30	
Oxygen	28	224	34·48	
Lime	2	56	8·61	8·32
		650	100·00	

It appears, therefore, that the decomposition of the substance had advanced in this case still further even than in the last, as it contained 2 equivs. of water less.

The last specimen I examined was one which was prepared in the usual manner by precipitation with alcohol, then mixed with water and allowed to decompose until bubbles of gas began to be disengaged, and the smell peculiar to this substance when in a state of putrefaction began to be emitted, then treated with boiling alcohol until nothing more was dissolved, and dried.

0·4630 grm., obtained in this manner and burnt with oxide of copper and chlorate of potash, gave 0·7260 carbonic acid and 0·1760 water.

0·5270 grm. gave 0·2705 chloride of platinum and ammonium.

0·2990 grm. gave on being incinerated 0·0720 carbonate of lime, containing 0·04040 lime =13·51 per cent. After treatment with sulphuric acid, this yielded 0·0980 sulphate of lime, containing 0·04042 lime =13·52 per cent.

In 100 parts it therefore consisted of—

Carbon	45·65
Hydrogen	4·22
Nitrogen	3·22
Oxygen	33·40
Lime	13·51

The only formula with which this composition agrees, and which is at the same time in accordance with the preceding formulæ, is $C_{52}H_{28\frac{1}{2}}N_{1\frac{1}{2}}O_{28}+3CaO$, which requires in 100 parts—

Carbon	46·60
Hydrogen	4·25
Nitrogen	3·13
Oxygen	33·48
Lime	12·54

From this formula it may be inferred, that during the further progress of decomposition erythrozym loses ammonia in addition to carbonic acid and water, for

$$C_{52}H_{28\frac{1}{2}}N_{1\frac{1}{2}}O_{28}+4CO_2+4HO+\tfrac{1}{2}NH_3=C_{56}H_{34}N_2O_{40}.$$

I shall conclude this part of my paper with a few remarks on the *vexata quæstio* of the existence or non-existence of more than one colouring matter in the madder root. Robiquet, the discoverer of alizarine, attributed the tinctorial power of madder partly to that substance and partly to another, on which he bestowed the name of purpurine. Gaultier de Claubry and Persoz were of opinion that there are two colouring matters in madder. Runge has described three substances obtained by him from madder, all of which he considers as colouring matters playing a part in the process of madder dyeing. Schiel, Debus and Wolff and Strecker all assume the existence of two distinct colouring matters. Notwithstanding, however, the array of authority in favour of this view, I have never been able to convince myself that the entire tinctorial power of madder was not due to alizarine alone, and that consequently all substances derived from madder, if endowed with any such power, owe it to their containing alizarine; and after having isolated and examined the primitive substance, from which all the bodies in madder endowed with a red or yellow colour, or capable of producing these colours, are derived, and after having exposed this substance to

the influence of all the reagents with which madder itself under ordinary circumstances is brought into contact, I find myself entirely confirmed in my opinion. I should indeed think it unnecessary to add anything confirmatory of the conclusions which must suggest themselves at once from the perusal of the preceding pages, but as Messrs. Wolff and Strecker have made it one of the special objects of their investigation to prove the existence of more than one colouring matter in madder, I think it may not be out of place here to add a few remarks to show how, in my opinion, these chemists, as well as their predecessors, have been misled, and also to give an account of some experiments still further confirmatory of the opinion which I have always held.

The second colouring matter, which, according to the chemists just mentioned, exists in madder in addition to alizarine, and which has received at various times the names of purpurine, madder-purple, and oxylizaric acid, possesses, according to those observers, the property of being easily soluble with a red colour in boiling alum liquor. Wolff and Strecker assert that this substance is peculiarly a product of fermentation, and in order to prepare it, they mix madder with water and yeast, allow the mixture to stand in a warm place until the effervescence produced by fermentation has ceased, and the liquid has acquired a strong acid reaction and contains alcohol, after which they strain the liquid through a cloth, wash the mass on the cloth with water, and then treat it with boiling alum liquor. From the bright red solution a substance separates on cooling in red flocks, which, as well as the orange-coloured flocks produced by adding sulphuric acid to the liquid, consist, according to them, entirely of purpurine without any trace of alizarine. They purify it by crystallization from alcohol. Now I have shown above that the fermentation of madder, which is in fact synonymous with the fermentation of rubian, is due to the action of a peculiar substance, which I have called erythrozym, on rubian; that the action of this substance is very rapid; that it is not accompanied by any disengagement of gas; that it is terminated long before any effervescence or any acid reaction of the liquid begins to appear; that the products of the action do not differ essentially from those due to the action of acids and alkalies; that the formation of alizarine in about the same proportion as when acids or alkalies are employed is one of the results of the process, and that yeast exerts no decomposing power on rubian whatever. Hence it necessarily follows, that if the purpurine of Wolff and Strecker be not found as such among the products of the fermentation of rubian, it must consist of a mixture of two or more of those products.

Now I have mentioned in the first part of this paper, that though alizarine and verantine are both perfectly insoluble in boiling alum liquor when acted on separately, yet that when a mixture of both is employed, the mixture is found to be soluble in alum liquor with the colour characteristic of purpurine. Hence I concluded that purpurine is in fact a mixture of those two substances, a view with which all that is mentioned regarding purpurine completely coincides. I may mention

incidentally, that in making this experiment it is necessary to treat the verantine with a little dilute nitric acid, in order to destroy the alizarine which usually accompanies it, and then to remove the acid by washing with water before employing it, and that it generally succeeds best when a large excess of alizarine is used.

To this synthetical proof of the opinion here advocated, I will now add a few analytical ones. I will show, in the first place, that purpurine prepared in the manner mentioned by WOLFF and STRECKER is a substance of very variable composition, but that the variations in its composition may be easily explained by supposing it to consist of alizarine and verantine in different proportions; and secondly, that by treatment with nitric acid purpurine yields unchanged verantine and an acid, which is identical with that formed by the action of nitric acid on alizarine.

In the course of my investigation I obtained at the termination of the process for separating the products of the fermentation of rubian, an alcoholic liquid, from which the verantine had been deposited, but still containing a substance, which, from its solubility in alum liquor, would by most chemists be called purpurine. This substance was precipitated from the solution with water. Its colour was brownish-yellow. It was treated with boiling alum liquor, to which it communicated a bright red colour. The liquid was filtered boiling hot, and deposited on cooling a quantity of red flocks. The residue was treated with fresh quantities of alum liquor, until on cooling very few flocks separated. A great proportion of the substance employed remained undissolved. The flocks deposited from the alum liquor were collected on a filter, and washed with water in order to remove all the alum. After drying they formed a dark reddish-brown powder, which was almost entirely soluble in alcohol. The alcoholic solution left on evaporation a bright red mass, in which no trace of anything crystalline was discernible. Its analysis gave the following results:—

0·4820 grm. gave 1·1240 carbonic acid and 0·1800 water.

0·8060 grm. left on being incinerated 0·0310 alumina=3·84 per cent.

After making the proper correction for the alumina, the quantity of which, in relation to that of the other constituents, seems to be indefinite, these numbers correspond in 100 parts to—

Carbon	66·13
Hydrogen	4·31
Oxygen	29·56

This composition does not differ very widely from that given for oxylizaric acid by DEBUS.

Now the formula $C_{56}H_{20}O_{19}=C_{14}H_5O_4+3C_{14}H_5O_5$ requires in 100 parts—

Carbon	66·14
Hydrogen	3·93
Oxygen	29·93

A quantity of material similar to the last, obtained on a different occasion, was treated in the same way with boiling alum liquor, and the deposit formed on the liquor cooling was submitted to analysis. It had a much lighter colour than the preceding, and when dissolved in alcohol, the latter left on evaporation crystals, apparently of alizarine, mingled with red crystalline masses resembling impure alizarine.

0·4000 grm. gave 0·9870 carbonic acid and 0·1390 water.

0·3840 grm. left on being incinerated 0·0060 alumina=1·56 per cent.

After making the necessary correction for the alumina, these numbers correspond in 100 parts to—

Carbon	68·36
Hydrogen	3·92
Oxygen	27·72

The formula $C_{56}H_{20}O_{17}=3C_{14}H_5O_4+C_{14}H_5O_5$ requires in 100 parts—

Carbon	68·29
Hydrogen	4·06
Oxygen	27·65

These two specimens therefore of a substance prepared in the same way, both of which would, according to the definition of WOLFF and STRECKER, pass for purpurine, possessed a composition, which in the one case corresponded to a mixture of 1 equiv. of alizarine and 3 equivs. of verantine, in the other case to a mixture of 3 equivs. of alizarine and 1 equiv. of verantine. A still more manifest proof of the fact of purpurine not being a substance of uniform composition, is derived from an examination of the liquid from which these specimens were deposited. To the bright red liquid from which the last was deposited, I added after filtration muriatic acid and boiled. A yellow precipitate was produced, which was collected on a filter and washed. A small quantity of it being dissolved in alcohol, the alcohol left on evaporation crystals of apparently pure alizarine. Its analysis also showed that it consisted of alizarine almost in a state of purity.

0·2390 grm. gave 0·6040 carbonic acid and 0·0980 water.

In 100 parts it contained therefore—

		Alizarine.
Carbon	68·92	69·42
Hydrogen . . .	4·55	4·13
Oxygen	26·53	26·45

I now prepared some so-called purpurine from garancine. The garancine was treated with boiling alum liquor, and the liquor was strained boiling hot through calico. On cooling there was formed a copious deposit, which was redissolved in fresh alum liquor. The deposit formed this time, which was very trifling, was

separated, and the substance contained in the liquid was precipitated with muriatic acid. The precipitate was yellow. Its analysis proved it to be almost pure verantine.

0·3685 grm. gave 0·8760 carbonic acid and 0·1400 water.

In 100 parts it contained therefore—

		Verantine.
Carbon	64·83	65·11
Hydrogen . . .	4·22	3·87
Oxygen	30·95	31·02

It appears therefore that the substance called purpurine cannot even be called a compound of alizarine and verantine, for it consists sometimes of one alone, sometimes of the other, sometimes of a variable mixture of both.

I now treated some purpurine, made in the same manner as the last, with boiling dilute nitric acid. Nitrous acid was disengaged, the bulk of the substance diminished very much, and its colour became lighter. After the action was completed, I allowed the liquid to cool, added water, collected the yellow flocks on a filter, and washed them with water to remove all the acid. After drying their colour was brownish-yellow, similar to that of pure verantine.

0·3820 grm. gave 0·9070 carbonic acid and 0·1240 water.

In 100 parts—

Carbon	64·72
Hydrogen	3·60
Oxygen	31·68

Its composition therefore, with the exception of a slight deficiency in the amount of hydrogen, was that of verantine.

The acid liquid filtered from the substance was evaporated almost to dryness, when it yielded a quantity of yellow crystals. These were washed with cold water, and then redissolved in a little boiling water. The boiling solution was decolorized with animal charcoal, and after being filtered boiling hot deposited on cooling a quantity of colourless crystals, having the appearance, and as their analysis showed, the composition of LAURENT's naphthalic acid.

0·5230 grm. gave 1·1020 carbonic acid and 0·1770 water.

In 100 parts—

		Napthalic acid.
Carbon	57·46	57·83
Hydrogen . . .	3·76	3·61
Oxygen	38·78	38·56

WOLFF and STRECKER mention, that purpurine yields this acid when subjected to the action of nitric acid, but the verantine which is found in an undecomposed state, after the action of the nitric acid has ceased, seems to have eluded their observation.

From these experiments I infer that purpurine, madder-purple, and the various similar bodies derived from madder, owe their property as colouring matters to an admixture of alizarine, and that they simply consist of the latter substance in a state of impurity.